PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Douglas N. Ishii

Serial No.: 08/571,802

Filing Date: December 13, 1995

For: METHOD FOR EFFECTING CHANGES IN THE CENTRAL NERVOUS SYSTEM BY ADMINISTRATION OF IGF-I OR IGF-II

Attorney Docket: 10606.0019.CNUS01

CERTIFICATE OF MAILING
37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: MAIL STOP APPEAL BRIEF--PATENTS, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22315-1450, on the date below:

12/1/03 [signature]
Date Signature

APPEAL BRIEF

MAIL STOP APPEAL BRIEF -- PATENTS
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicant submits an original and two copies of this Appeal Brief to the Board of Patent Appeals and Interferences in response to the final Office Action dated February 7, 2003, and in view of the Notice of Appeal filed May 29, 2003.

I. REAL PARTY IN INTEREST

The real party in interest for this application is the Colorado State University Research Foundation.

II. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences related to this application.

12/04/2003 AWONDAF1 00000069 012508 08571802
01 FC:2402 165.00 DA

III. STATUS OF THE CLAIMS

Claims 24-71 are pending in the present application. All of these claims were rejected in the final Office Action dated February 7, 2003 and the Office Action dated December 8, 1999.

IV. STATUS OF AMENDMENTS

All amendments submitted for this application have been entered.

V. SUMMARY OF THE INVENTION

The present invention is directed to a method for treating a mammal suffering from an injury to the central nervous system comprising parenteral nonintracranial administration of an IGF in an amount effective to treat the traumatic injury, wherein the IGF consists essentially of an amino acid sequence of a naturally occurring IGF.

VI. ISSUES ON APPEAL

1. Whether claims 24-71 are enabled under 35 U.S.C § 112, first paragraph.
2. Whether claims 24-71 meet the written description requirement of 35 U.S.C. § 112, first paragraph.
3. Whether claims 24-71 contain new matter.
4. Whether claims 24-71 meet the definiteness requirement under 35 U.S.C. § 112, second paragraph.
5. Whether claims 24-71 are anticipated under 35 U.S.C. § 102(e) over Lewis et al. (U.S. Patent No. 5,093,317).

VII. GROUPING OF THE CLAIMS

Claims 24-71 stand or fall together.

VIII. ARGUMENT

A. Rejections under 35 U.S.C. § 112, first paragraph

In the Office Action dated February 7, 2003, paragraph 5, the Examiner states that the Declaration of Dr. Douglas N. Ishii under 37 C.F.R. § 1.132 is insufficient to overcome the rejection of claims 24-71 based upon 35 U.S.C. § 112, first paragraph, because the Declaration is unsigned. Applicant respectfully submits that a signed copy of the Ishii Declaration was filed with a Supplemental Response Enclosing Executed Declaration of Douglas N. Ishii on November 16, 1998 (Exhibit A), as evidenced by the postcard bearing the stamp of Technology Center 1600/2900 (last two pages of Exhibit A). Accordingly, this rejection should be withdrawn.

In the Office Action dated February 7, 2003, paragraph 7, claims 24-71 are also rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to "convey to one skilled in the relevant art that the inventor(s) . . . had possession of the claimed invention." The Examiner characterizes this rejection as a written description and new matter rejection. According to the Examiner, the definitions for "IGF-I" and "IGF-2" provided in the specification are generic and do "not provide support for the subgeneric limitations claimed [i.e., the limitations to IGF's consisting essentially of amino acid sequences of naturally occurring IGF's]."

Applicant disagrees with the Examiner's characterization of the "naturally occurring" amendment. Applicant did not submit these amendments to change the scope of the IGF claim limitations from genus to subgenus. Rather, Applicant submitted this amendment to clarify "that the scope of the IGF in applicant's claims does not include the 'functional derivatives' of Lewis et al." (Response to Office Action dated April 7, 2000, p. 5.)

Applicant has already argued during the prosecution of this application that the scope of the IGF claim limitation does not include functional derivatives of IGF's, including fragments of

IGF's, analogs of IGF's, or analogs of fragments of IGF's. (Response to Office Action, April 7, 2000, pp. 4-5.) This argument is wholly consistent with the definitions of IGF-I and IGF-II found in the specification. (Specification at page 5, line 20, to page 6, line 9.) Thus, when Applicant amended the claims to include the term "naturally occurring", the scope of the claims did not change. Instead, these amendments clarified that scope.

Moreover, when Applicant amended the claims to recite "naturally occurring" IGF's, no new matter was added. Persons skilled in the art would understand that functional derivatives of IGF's are not naturally occurring IGF's. Furthermore, it would be clear to persons skilled in the art that IGF-I and "homologs of IGF-I from various animal species, whether extracted from tissues or derived as products of recombinant genetic expression vectors, and IGF molecules with substantial sequence homology to human and animal IGF-I that bind to type I IGF receptors" are all naturally occurring forms of IGF-I. (Specification at page 5, line 20 to line 28.) Likewise, it would be clear to persons skilled in the art that IGF-II and "homologs of IGF-II from various animal species, whether extracted from tissues or derived as products of recombinant genetic expression vectors, and IGF molecules with substantial sequence homology to human and animal IGF-II that bind to type I or type II IGF receptors" are all naturally occurring forms of IGF-II. (Specification at page 6, line 1 to line 9.)

Therefore, persons of ordinary skill in the art would understand the clear "naturally occurring" amendment language to be a short-hand version for the IGF's defined in the specification. Accordingly, the rejection directed to the "naturally occurring IGF" language should be withdrawn.

The Examiner also based the written description and new matter rejection on his assertion that the "claims encompass a subgenus of 'naturally occurring allelic variants' which is not

disclosed in the specification." (Office Action dated February 7, 2003, ¶ 7.) Applicant respectfully disagrees. As mentioned previously, IGF-I and IGF-II are defined in the specification. (Specification at page 5, line 20, to page 6, line 9.) The specification and examples of the application refer repeatedly to IGF-I and IGF-II. Thus, persons of skill in the art would understand that the inventors, at the time the application was filed, had possession of naturally occurring IGF's.

Moreover, naturally occurring IGF's are well known in the art. (*See, e.g.*, CONCISE ENCYCLOPEDIA BIOCHEMISTRY 305-307 (Thomas Scott & Mary Eagleson eds., 2nd ed. 1988) (Exhibit B).) IGF's have been defined as "a family of polypeptides present in vertebrate blood that share considerable structural and functional similarity with insulin, but are distinguishable from it by a lack of immunological cross reactivity." (*Id.*) IGF-I and IGF-II have well known structures and functions. (*See id.*) (showing the amino acid structures of human IGF-I and IGF-II).

Finally, the Examiner's reliance on *University of California v. Eli Lilly & Co.*, 119 F.3d 1559 (Fed. Cir. 1997), is misplaced. (*See* Office Action dated February 7, 2003, p. 3.) Since that decision, the Federal Circuit has decided that the rule from *Eli Lilly* does not apply unless the "claim terms at issue [] are [] new or unknown biological materials that ordinarily skilled artisans would easily miscomprehend." (*Amgen Inc. v. Hoescht Marion Roussel, Inc.*, 314 F.3d 1313, at 1332 (Fed. Cir. 2003).) Unlike the novel insulin DNA sequences at issue in *Eli Lilly*, IGF's were well known to persons of skill in the art. Thus, *Eli Lilly* does not apply. Instead, since the specification describes and defines IGF's, and since persons of skill in the art understand the functional and structural characteristics of IGF's, the "allelic variant" basis of the Examiner's written description and new matter rejection cannot stand.

The Examiner's final basis for the written description and new matter rejection is his assertion that the terms "parenteral nonintracranial administration" are not disclosed in the specification. (Office Action dated February 7, 2003, p. 4.) Applicant respectfully disagrees. In the background section of the specification, Applicant clearly states that "there is a need in the art of biotechnology and the biopharmaceutical industries for a method of effecting changes to the central nervous system by the administration of large protein molecules across the blood-brain barrier and blood spinal cord barrier, particularly where the method of treatment involves administration of IGF-I or IGF-II." (Specification page 4, line 25, to page 5, line 2.) Other information included in the background section concerned the transport of large protein molecules, such as IGF-I and IGF-II, across the blood brain barrier and blood spinal cord barrier, including a discussion concerning the "very tight junctions" between the endothelial cells of the blood brain barrier limited transport of molecules. (Specification page 2, line 10, to page 3, line 23.) The background section discussed how intracranial administration was "invasive" and "difficult, risky and require[d] costly surgical procedure." (Specification page 3, line 9 to line 14.)

The Detailed Description portion of the specification further describes the present invention as "all outside the blood-brain barrier or blood-spinal cord barrier." (Specification at page 9, line 12 to line 14.) Moreover, in the examples, rats were "implanted subcutaneously in the mid-back with miniosmotic pumps releasing either recombinant human IGF-I (4.8 μg per day) or vehicle." (Specification at page 11, line 11 to line 12.) This administration method is clearly a parenteral nonintracranial type of administration. Accordingly, this rejection should be withdrawn.

B. Rejection of claims under 35 U.S.C. § 112, second paragraph

In the Office Action dated February 7, 2003, paragraph 6, claims 24-71 are also rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner asserts that the terms IGF-I and IGF-II consisting "essentially of an amino acid sequence of a naturally occurring" IGF-I or IGF-II are "confusing and ambiguous" and that the terms IGF-I and IGF-II "appear to be overlapping such that it is not clear when an IGF-I molecule homolog is not an IGF-II homolog molecule." (Office Action dated February 7, 2003, p. 2.)

Applicant contends that these terms are not confusing and ambiguous, rather they are clear to a person having ordinary skill in the art. The CONCISE ENCYCLOPEDIA BIOCHEMISTRY makes clear the distinct differences between IGF-I and IGF-II, and provides well-known structures of the human forms of these compounds. (CONCISE ENCYCLOPEDIA BIOCHEMISTRY 305-307 (Thomas Scott & Mary Eagleson eds., 2nd ed. 1988) (Exhibit B)). Because the differences are well known in the art there is no need to rehash these differences in the specification of the patent application. The definitions of IGF-I and IGF-II are clear and a person having skill in the art would know the difference between the two compounds. For this reason, applicant respectfully requests that the rejection on the ground of indefiniteness be withdrawn.

C. Rejection of claims under 35 U.S.C. § 102(e)

In the Office Action dated February 7, 2003 (as well as previous Office Actions), claims 24-71 are rejected under 35 U.S.C. § 102(e) as being anticipated by Lewis et al. The Examiner asserts that "Lewis et al. not only teach intracranial administration to overcome the blood brain barrier but also teach the parenteral administration of IGFs which by definition is

nonintracranial. Thus, Lewis et al. teach both parenteral and intracranial administration." (Office Action mailed December 8, 1999, ¶ 9.)

Applicant strongly disagrees with these assertions. Lewis et al. simply does not teach treatment of the brain by parenteral nonintracranial administration. Instead, Lewis discloses the treatment of the brain by intracranial administration, which means that a hole must be made in the skull and that the treatment of IGF is conducted through the hole in the skull. Lewis teaches away from parenteral nonintracranial administration of a nonmodified IGF by asserting that a naturally-occurring IGF does not cross the blood-brain barrier:

> Where the polypeptide is intended for use as a therapeutic for disorders of the CNS, an additional problem must be addressed: overcoming the so-called "blood-brain barrier," the brain capillary wall structure that effectively screens out all but selected categories of molecules present in the blood, preventing their passage into the brain. While the blood-brain barrier may be effectively bypassed by direct infusion of the polypeptide into the brain, the search for a more practical method has focused on enhancing transport of the polypeptide of interest across the blood-brain barrier, such as by making the polypeptide more lipophilic, by conjugating the polypeptide of interest to a molecule which is naturally transported across the barrier, or by reducing the overall length of the polypeptide chain.

Lewis et al., col. 3, lines 44-58.

Even the Examples of Lewis are limited to either *in vitro* experiments or administration of modified IGF to rat models via a hole in the skull. More particularly, the Lewis et al. examples address *in vitro* methods for measuring effectiveness of IGF treatment (Examples 1-3), administration of IGF by intracerebral injection (*i.e.*, injection through a hole in the skull) (Examples 4 and 5), methods for modifying IGF's (Examples 6-10), and an *in vitro* method for measuring the effectiveness of the IGF modifications (Example 11). Nowhere does Lewis et al. teach or even suggest the administration of IGF's via a parenteral nonintracranial method. On the contrary, Lewis et al. emphatically and unmistakably teach that the IGF must either be

modified or be delivered intracranially. Therefore, Lewis et al. does not teach or suggest a method of administering IGFs that is both parenteral and nonintracranial.

The Examiner also asserts that "[applicant's] claimed limitation directed to IGF is the same scope as the IGF claimed which includes functional derivatives and unmodified IGF." (Office Action mailed December 8, 1999, p. 2.) That statement is not correct, as the scope of the IGF in applicant's claims does not include the "functional derivatives" of Lewis et al. Lewis et al. alleges that diseases such as Alzheimer's and Parkinson's are treated by "administering to the animal an effective amount of a functional derivative, e.g. a fragment or analog of IGF-I or of IGF-II" (column 4, lines 3-5; emphasis added). A method for enhancing the cholinergic activity of cholinergic neuronal cells is alleged to be accomplished by "administering to the mammal an effective amount of a functional derivative of IGF-I or IGF-II, preferably a fragment of IGF-I, of IGF-II or, alternatively, an analog of IGF-I , of IGF-II, or of a fragment of IGF-I or IGF-II" (column 4, lines 16-20; emphasis added). Furthermore, Lewis et al. recite that "[t]he method of the invention uses functional derivatives of IGF-I and of IGF-II to enhance the survival rate and/or the cholinergic activity of mammalian cells at an increased risk of death due to some factor such as disease, injury, or natural aging process" (column 4, lines 31-35; emphasis added). Finally, Lewis et al. specifically teach that their "invention is directed to the modification of neuroactive polypeptides such as IGF-I and IGF-II and their functional derivatives" (column 5, lines 60-62; emphasis added).

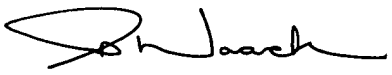
Therefore, the rejection under 35 U.S.C. § 102(e) based on Lewis et al. should be withdrawn.

IX. CONCLUSION

In view of the foregoing arguments, applicant respectfully requests reversal of the rejections under 35 U.S.C. § 112, first and second paragraphs, and 35 U.S.C. § 102 (e) based on Lewis et al.

The fee for filing an Appeal Brief ($165.00), and any other fee due in connection with this Appeal Brief, should be charged to Howrey Simon Arnold & White Deposit Account No. 01-2508/10606.0019.00US01/WAA.

Respectfully submitted,



Janelle D. Waack
Reg. No. 36,300

Attorney for Assignee
COLORADO STATE UNIVERSITY
RESEARCH FOUNDATION

HOWREY SIMON ARNOLD & WHITE, LLP
750 Bering Drive
Houston, Texas 77057-2198
(713) 787-1686

December 1, 2003

APPENDIX

Claims Pending in U.S. Patent Application Serial No. 08/571,802

24. A method for treating a mammal suffering from traumatic injury to the central nervous system comprising parenteral nonintracranial administration of an IGF-I in an amount effective to treat the traumatic injury, wherein the IGF-I consists essentially of an amino acid sequence of a naturally occurring IGF-I.

25. The method of claim 24, wherein IGF-I is administered in an amount from about 0.1 μg/kg body weight/day up to about 4 mg/kg body weight/day.

26. The method of claim 24, wherein the mammal is a human.

27. The method of claim 24, wherein the traumatic injury is to the brain.

28. The method of claim 24, wherein the traumatic injury is to the spinal cord.

29. A method for treating a mammal suffering from traumatic injury to the central nervous system comprising parenteral nonintracranial administration of an IGF-II in an amount effective to treat the traumatic injury, wherein the IGF-II consists essentially of an amino acid sequence of a naturally occurring IGF-II.

30. The method of claim 29, wherein IGF-II is administered in an amount from about 0.1 μg/kg body weight/day up to about 4 mg/kg body weight/day.

31. The method of claim 29, wherein the mammal is a human.

32. The method of claim 29, wherein the traumatic injury is to the brain.

33. The method of claim 29, wherein the traumatic injury is to the spinal cord.

34. A method for treating a mammal suffering from a stroke comprising parenteral nonintracranial administration of an IGF-I in an amount effective to treat the stroke, wherein the IGF-I consists essentially of an amino acid sequence of a naturally occurring IGF-I.

35. The method of claim 34, wherein IGF-I is administered in an amount from about 0.1 μg/kg body weight/day up to about 4 mg/kg body weight/day.

36. The method of claim 34, wherein the mammal is a human.

37. A method for treating a mammal suffering from a stroke comprising parenteral nonintracranial administration of an IGF-II in an amount effective to treat the stroke, wherein the IGF-II consists essentially of an amino acid sequence of a naturally occurring IGF-II.

38. The method of claim 37, wherein IGF-II is administered in an amount from about 0.1 μg/kg body weight/day up to about 4 mg/kg body weight/day.

39. The method of claim 37, wherein the mammal is human.

40. A method for treating a mammal suffering from traumatic brain injury or stroke comprising increasing the circulating concentration of an IGF-I to a concentration effective to treat the traumatic brain injury or stroke; wherein increasing the circulating concentration of IGF-I is accomplished by parenteral nonintracranial administration of IGF-I, wherein the IGF-I consists essentially of an amino acid sequence of a naturally occurring IGF-I.

41. The method of claim 40, wherein the mammal is a human.

42. The method of claim 40, wherein the circulating IGF-I concentration is increased by administering IGF-I in an amount from about 0.1 μg/kg body weight/day up to about 4 mg/kg body weight/day.

43. A method for treating a mammal suffering from traumatic brain injury or stroke comprising increasing the circulating concentration of an IGF-II to a concentration effective to

treat the traumatic brain injury or stroke; wherein increasing the circulating concentration of IGF-II is accomplished by parenteral nonintracranial administration of IGF-II, wherein the IGF-II consists essentially of an amino acid sequence of a naturally occurring IGF-II.

44. The method of claim 43, wherein the mammal is a human.

45. The method of claim 43, wherein the circulating IGF-I concentration is increased by administering IGF-II in an amount from about 0.1 μg/kg body weight/day up to about 4 mg/kg body weight/day.

46. A method for treating damaged locus ceruleus neurons or axons in a mammal, comprising parenteral nonintracranial administration of an IGF in an amount effective to treat the locus ceruleus neurons or axons, wherein the IGF consists essentially of an amino acid sequence of a naturally occurring IGF.

47. The method of claim 46, wherein the IGF is IGF-I.

48. The method of claim 47, wherein IGF-I is administered in an amount from about 0.1 μg/kg body weight/day up to about 4 mg/kg body weight/day.

49. The method of claim 47, wherein the mammal is a human.

50. The method of claim 47, wherein the locus cereleus is damaged due to traumatic injury.

51. The method of claim 47, wherein the locus cereleus is damaged due to stroke.

52. The method of claim 46, wherein the IGF is IGF-II.

53. The method of claim 52, wherein IGF-II is administered in an amount from about 0.1 μg/kg body weight/day up to about 4 mg/kg body weight/day.

54. The method of claim 52, wherein the mammal is a human.

55. The method of claim 52, wherein the locus cereleus is damaged due to traumatic injury.

56. The method of claim 52, wherein the locus cereleus is damaged due to stroke.--

57. A method for treating injury to the central nervous system in a mammal comprising parenteral nonintracranial administration of an IGF in an amount effective to treat the injury, wherein the IGF consists essentially of an amino acid sequence of a naturally occurring IGF.

58. The method of claim 57, wherein the IGF is IGF-I

59. The method of claim 58, wherein the IGF-I is administered in an amount from about 0.1μg/kg body weight/day up to about 4 mg/kg body weight/day.

60. The method of claim 58, wherein the mammal is a human.

61. The method of claim 58, wherein the central nervous system is damaged due to traumatic injury.

62. The method of claim 58, wherein the central nervous system is damaged due to stroke.

63. The method of claim 57, wherein the IGF is IGF-II.

64. The method of claim 63, wherein the IGF-II is administered in an amount from about 0.1μg/kg body weight/day up to about 4 mg/kg body weight/day.

65. The method of claim 63, wherein the mammal is a human.

66. The method of claim 63, wherein the central nervous system is damaged due to traumatic injury.

67. The method of claim 63, wherein the central nervous system is damaged due to stroke.

68. The method of claim 46, wherein the locus ceruleus is damaged due to Parkinson's disease.

69. The method of claim 52, wherein the locus ceruleus is damaged due to Parkinson's disease.

70. The method of claim 58, wherein the locus ceruleus is damaged due to Parkinson's disease.

71. The method of claim 63, wherein the locus ceruleus is damaged due to Parkinson's disease.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DOUGLAS N. ISHII

Serial No.: 08/571,802

Filed: February 17, 1998

For: METHOD FOR TREATING STROKE OR TRAUMATIC INJURY TO THE CENTRAL NERVOUS SYSTEM WITH IGF-I OR IGF-II

Group Art Unit: 1646

Examiner: M. Pak

Attorney Docket: CSUA019--1/WAA

SUPPLEMENTAL RESPONSE ENCLOSING EXECUTED DECLARATION OF DOUGLAS N. ISHII

VIA HAND DELIVERY

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

Enclosed is an executed Declaration of Douglas N. Ishii to be considered with the Amendment and Remarks filed on November 2, 1998. An unexecuted version of the Declaration was filed with that Amendment and Remarks.

Please note that the executed Declaration has the following changes from the text of the unexecuted Declaration: the numbering of the Exhibits was revised to match the appendix, at page 2, line 5, the word "destroyed" was replaced with "damaged" and at page 6, lines 12-13, the term "potential adsorption of radioactive IGF" was replaced with "potential nonspecific adsorption of radioactive IGF to assay tubes".

If any fees are required, the Assistant Commissioner is authorized to deduct those fees from Arnold, White & Durkee Deposit Account No. 01-2508/CSUA019--1WAA.

Respectfully submitted,

November 13, 1998

Janelle D. Waack
Reg. No. 36,300

ARNOLD, WHITE & DURKEE
P. O. Box 4433
Houston, Texas 77210-4433
(713) 787-1686

ATTORNEY FOR ASSIGNEE,
COLORADO STATE UNIVERSITY
RESEARCH FOUNDATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DOUGLAS N. ISHII

Serial No.: 08/571,802

Filed: February 17, 1998

For: METHOD FOR TREATING STROKE OR TRAUMATIC INJURY TO THE CENTRAL NERVOUS SYSTEM WITH IGF-I OR IGF-II

Group Art Unit: 1646

Examiner: M. Pak

Attorney Docket: CSUA019--1/WAA

DECLARATION OF DOUGLAS N. ISHII UNDER 37 C.F.R. § 1.132

I, Douglas N. Ishii, declare:

1. I am a professor at the Colorado State University College of Veterinary Medicine and Biomedical Sciences and have held that position since 1989. I have been studying and working with neurotropic factors since 1974.

2. I am the inventor of the subject matter that is claimed and for which a patent is sought on the invention titled "Method for Treating Stroke or Traumatic Injury to the Central Nervous System with IGF-I or IGF-II." I have reviewed and understand the contents of this application.

3. I understand that the specification is objected to and claims are rejected under 35 U.S.C. § 112, first paragraph, based on the enablement requirement. The following information indicates that the specification does enable the pending claims.

4. The Locus Ceruleus Model in Example III of the Specification

Example III of the specification, which was conducted by me or under my supervision, demonstrated that chemically-induced damage to the locus ceruleus can be ameliorated by parenteral administration of IGF-II. In that example, Sprague Dawley rats were inject with 6-hydroxydopamine to damage noradrenergic locus ceruleus cells in the pons of the brain. The location of the locus ceruleus cells and axons is depicted in Kandel et al., *Principles of Neural Science*, 3d ed. (1991) p. 694 (Ex. A). When these cells were damaged, the associated axons, which descend down the spinal cord and synapse on interneurons, were destroyed as manifested by a loss in the duration and amplitude of the hind limb reflex force. Lesioned rats that were parenterally administered IGF-II, however, were significantly spared from loss of hind limb reflex force. Example II of the specification also demonstrated that IGF-I effectively ameliorated impairment of the central nervous system by diabetes.

5. Additional Data for the Locus Ceruleus Model with IGF-I

The following experiment demonstrates that parenteral administration of IGF-I ameliorates chemically-induced damage to the locus ceruleus. This experiment was conducted in the same manner as Example III, except that IGF-I was used in place of IGF-II and the results were based on the measurements of groups of rat subjects. Sprague Dawley rats were treated with 6-hydroxydopamine to lesion the noradrenergic axons arising from the brainstem and descending down the spinal cord. Following lesioning, rats were implanted with subcutaneous pumps that released either vehicle or IGF-I for 1 week, and the hind limb withdrawal reflex force was measured after 3 weeks. Fig. 1 (Ex. B) shows the mean maximal force of hind-limb reflex in various groups of rats: NL, nonlesioned rats; L + Veh, lesioned rats treated with subcutaneous

pumps releasing vehicle; L + IGF-I, lesioned rats treated with subcutaneous pumps releasing IGF-I (4.8 ug/rat/day). The means + SEM (standard estimate of the mean) are shown for each group. *P < 0.009 (L + IGF-I) vs. (L + Veh); **P < 0.0002 (NL) vs. (L + Veh). These data show that the mean force of hind-limb withdrawal was significantly reduced in lesioned versus nonlesioned rats. Moreover, this force was significantly preserved in IGF-I treated versus vehicle treated lesioned rats. Therefore, subcutaneous treatment with human IGF-I acted across the blood brain barrier to preserve function in a central nervous system lesioned mammal.

The spinal cords from these rats were subsequently sectioned to determine whether IGF treatment could preserve the noradrenergic circuitry in the lumbar region of the spinal cord containing the motoneurons involved in the hind limb reflex. Dopamine-ß-hydroxylase (DBH) is an enzyme in the pathway producing noradrenaline and can be used to identify the noradrenergnic axons in the spinal cord. Such axons are beaded with varicosities containing neurotransmitters. An anti-DBH antibody was tested and found to selectively stain the noradrenergic neurons in the locus ceruleus but not other cells in the surrounding area. Using this antibody, the noradrenergic axons and vesicles were detected in the lumbar region of the spinal cord of nonlesioned rats.

A total of six sections (30 μm sections taken every 5 mm) from the lumbar region of each rat of each treatment group (N - 7 or 8 rats per group) were subjected to morphometric analysis using the Metamorph system (Universal Imaging Corp., West Chester, PA). Fig 2 (Ex. C) shows a region of the spinal cord stained with antibody showing noradrenergic varicosities arranged like beads on axons. The boxed region is a standard area selected for analysis. Fig. 2B shows the varicosities selected in the standard area for computer analysis. The mean number of varicosities

per unit spinal cord area was calculated for each rat group. Figure 2C shows means + SEM for each group. *P < 0.01 for (L + IGF-I) vs. (L + Veh); **P < 0.0002, (L + Veh) vs. NL. These data show that the mean number of adrenergic varicosities was significantly reduced in lesioned rats. Moreover, the mean number of adrenergic varicosities was significantly preserved in IGF-I treated versus vehicle-treated lesioned rats. Thus, subcutaneously administered IGF can act across the blood brain barrier to prevent loss of spinal cord circuitry as well as preserve function in a mammal with a locus ceruleus lesion.

6. Scientific Publications Acknowledge Locus Ceruleus Damage in Neurodegenerative Trauma and Disorders

I have reviewed the scientific literature relating to locus ceruleus damage in neurodegenerative trauma and disorders. The locus ceruleus is involved in many diseases and disorders, which I believe are susceptible to treatment by the invention based on the teachings of the specification and the above-mentioned locus ceruleus model in rat. The following conditions are recognized as involving disease or damage to the locus ceruleus:

(a)	Alzheimer's Disease	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983) (Ex. D); Chan-Palay et al., *J. Comparative Neurology*, 287:373-392 (1989)(Ex. E);
(b)	Parkinson's Disease	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983); Chan-Palay et al., *J. Comparative Neurology*, 287:373-392 (1989);
(c)	Dementia pugilistica	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983);
(d)	Progressive supernuclear palsy	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983);
(e)	Huntington's Disease	Zweig et al., *Annals of Neurology* 24:233-242 (1988) (Ex. F);
(f)	Pick's Disease	Arima et al., *Acta Neuropathologica* 79:629-33, (1990) (Ex. G);

(g)	Major depression	Chan-Palay et al., *J. Comparative Neurology*, 287:373-392 (1989);
(h)	Suicide	Arango et al., *Biol. Psychiatry* 39:112-120 (1996) (Ex. H);
(i)	Schizophrenia	Chan-Palay et al., *J. Comparative Neurology*, 287:373-392 (1989);
(j)	Down's Syndrome	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983);
(k)	Shy-Drager Syndrome	Sima et al., *Clinical Neuropathology* 6:49-54 (1987) (Ex. I);
(l)	Rett Syndrome	Nomura et al., *Brain & Development* 7:334-341 (1985) (Ex. J); and
(m)	Olivopontocerebellar atrophy	Grijalba et al., *J. Neural Transmission* 96:135-42 (1994) (Ex. K).

7. Experiments Further Demonstrating Parenterally-Administered IGFs Crossing the Blood-Brain Barrier

An osmotic minipump containing human IGF-I was implanted subcutaneously in the mid-back region of a rat to continuously deliver human IGF-I. Cerebrospinal fluid (CSF) was removed from the rats prior to treatment (zero time) and after 0.5, 1, 2 and 3 days of human IGF-I infusion. The CSF was subjected to acid-ethanol extraction to dissociate IGF from IGF binding proteins. The human IGF-I in rat CSF was measured using a very sensitive ELISA assay that detected human IGF-I but not human IGF-II, insulin, rat IGF-I, rat IGF-II or rat insulin. The anti-IGF antibody was coupled to horse radish peroxidase, and tetramethylbenzidine substrate was used to produce a colored product that permitted detection of immunoreactive human IGF-I (ihIGF-I) at O.D. 450 nm.

Fig. 3 (Ex. L) shows that human IGF-I was measured in rat CSF after 0.5, 1, 2 and 3 days. It was not detected at 0 days, and the lack of signal confirmed that the assay did not detect rat IGF-I, IGF-II or insulin in rat CSF. These data show that human IGF-I administered subcutaneously can cross the blood brain barrier and enter the CSF.

In the second example, human ^{125}I-IGF-I was injected subcutaneously in a rat. After 2 hours, CSF (100 μl) was carefully withdrawn with a syringe mounted on a micromanipulator after surgical exposure of the atlanto-occipital membrane. Serum was also withdrawn. The treated rat CSF and serum, and other test samples, were subjected to polycrylamide gel electrophoresis in sodium dodecylsulfate-containing (SDS PAGE) gels. This gel separates proteins on the basis of size. Fig. 4 (Ex. M) shows a silver stain of the gel (upper panel) and autoradiogram of the same gel (lower panel). The lanes contained the following samples (from left to right): protein molecular weight markers; BSA buffer used to block potential nonspecific adsorption of radioactive IGF to assay tubes; standard ^{125}I-IGF-I plus CSF from untreated rat (shows normal migration position of standard ^{125}I-IGF-I is not influenced by mixing with CSF); standard ^{125}I-IGF-I plus BSA (shows normal migration position of ^{125}I-IGF-I); radioactive CSF from treated rat (shows ^{125}I-IGF injected subcutaneously crosses the BBB and has size indistinguishable from standard intact ^{125}I-IGF-I); standard nonradioactive human IGF-I (shows position of migration on silver-stained gel at 7.5 kDa size); and radioactive plasma from treated rat (shows ^{125}I-IGF-I administered subcutaneously accumulates in plasma to permit crossing the BBB).

Taken together, these data show that human IGF administered subcutaneously *in vivo* in a mammal can accumulate in serum and cross the blood brain barrier as intact IGF.

8. I further declare that all of these statements based on my knowledge are true and all statements made on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

11-2-98
Date

Dr. Douglas N. Ishii

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DOUGLAS N. ISHII

Serial No.: 08/571,802

Filed: February 17, 1998

For: METHOD FOR TREATING STROKE OR TRAUMATIC INJURY TO THE CENTRAL NERVOUS SYSTEM WITH IGF-I OR IGF-II

Group Art Unit: 1646

Examiner: M. Pak

Attorney Docket: CSUA019--1/WAA

SUPPLEMENTAL RESPONSE ENCLOSING EXECUTED DECLARATION OF DOUGLAS N. ISHII

VIA HAND DELIVERY

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

Enclosed is an executed Declaration of Douglas N. Ishii to be considered with the Amendment and Remarks filed on November 2, 1998. An unexecuted version of the Declaration was filed with that Amendment and Remarks.

Please note that the executed Declaration has the following changes from the text of the unexecuted Declaration: the numbering of the Exhibits was revised to match the appendix, at page 2, line 5, the word "destroyed" was replaced with "damaged" and at page 6, lines 12-13, the term "potential adsorption of radioactive IGF" was replaced with "potential nonspecific adsorption of radioactive IGF to assay tubes".

If any fees are required, the Assistant Commissioner is authorized to deduct those fees from Arnold, White & Durkee Deposit Account No. 01-2508/CSUA019--1WAA.

Respectfully submitted,

November 13, 1998

Janelle D. Waack
Reg. No. 36,300

ARNOLD, WHITE & DURKEE
P. O. Box 4433
Houston, Texas 77210-4433
(713) 787-1686

ATTORNEY FOR ASSIGNEE,
COLORADO STATE UNIVERSITY
RESEARCH FOUNDATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

DOUGLAS N. ISHII

Serial No.: 08/571,802

Filed: February 17, 1998

For: METHOD FOR TREATING STROKE OR TRAUMATIC INJURY TO THE CENTRAL NERVOUS SYSTEM WITH IGF-I OR IGF-II

Group Art Unit: 1646

Examiner: M. Pak

Attorney Docket: CSUA019--1/WAA

DECLARATION OF DOUGLAS N. ISHII UNDER 37 C.F.R. § 1.132

I, Douglas N. Ishii, declare:

1. I am a professor at the Colorado State University College of Veterinary Medicine and Biomedical Sciences and have held that position since 1989. I have been studying and working with neurotropic factors since 1974.

2. I am the inventor of the subject matter that is claimed and for which a patent is sought on the invention titled "Method for Treating Stroke or Traumatic Injury to the Central Nervous System with IGF-I or IGF-II." I have reviewed and understand the contents of this application.

3. I understand that the specification is objected to and claims are rejected under 35 U.S.C. § 112, first paragraph, based on the enablement requirement. The following information indicates that the specification does enable the pending claims.

4. The Locus Ceruleus Model in Example III of the Specification

Example III of the specification, which was conducted by me or under my supervision, demonstrated that chemically-induced damage to the locus ceruleus can be ameliorated by parenteral administration of IGF-II. In that example, Sprague Dawley rats were inject with 6-hydroxydopamine to damage noradrenergic locus ceruleus cells in the pons of the brain. The location of the locus ceruleus cells and axons is depicted in Kandel et al., *Principles of Neural Science*, 3d ed. (1991) p. 694 (Ex. A). When these cells were damaged, the associated axons, which descend down the spinal cord and synapse on interneurons, were destroyed as manifested by a loss in the duration and amplitude of the hind limb reflex force. Lesioned rats that were parenterally administered IGF-II, however, were significantly spared from loss of hind limb reflex force. Example II of the specification also demonstrated that IGF-I effectively ameliorated impairment of the central nervous system by diabetes.

5. Additional Data for the Locus Ceruleus Model with IGF-I

The following experiment demonstrates that parenteral administration of IGF-I ameliorates chemically-induced damage to the locus ceruleus. This experiment was conducted in the same manner as Example III, except that IGF-I was used in place of IGF-II and the results were based on the measurements of groups of rat subjects. Sprague Dawley rats were treated with 6-hydroxydopamine to lesion the noradrenergic axons arising from the brainstem and descending down the spinal cord. Following lesioning, rats were implanted with subcutaneous pumps that released either vehicle or IGF-I for 1 week, and the hind limb withdrawal reflex force was measured after 3 weeks. Fig. 1 (Ex. B) shows the mean maximal force of hind-limb reflex in various groups of rats: NL, nonlesioned rats; L + Veh, lesioned rats treated with subcutaneous

pumps releasing vehicle; L + IGF-I, lesioned rats treated with subcutaneous pumps releasing IGF-I (4.8 ug/rat/day). The means + SEM (standard estimate of the mean) are shown for each group. *P < 0.009 (L + IGF-I) vs. (L + Veh); **P < 0.0002 (NL) vs. (L + Veh). These data show that the mean force of hind-limb withdrawal was significantly reduced in lesioned versus nonlesioned rats. Moreover, this force was significantly preserved in IGF-I treated versus vehicle treated lesioned rats. Therefore, subcutaneous treatment with human IGF-I acted across the blood brain barrier to preserve function in a central nervous system lesioned mammal.

The spinal cords from these rats were subsequently sectioned to determine whether IGF treatment could preserve the noradrenergic circuitry in the lumbar region of the spinal cord containing the motoneurons involved in the hind limb reflex. Dopamine-ß-hydroxylase (DBH) is an enzyme in the pathway producing noradrenaline and can be used to identify the noradrenergnic axons in the spinal cord. Such axons are beaded with varicosities containing neurotransmitters. An anti-DBH antibody was tested and found to selectively stain the noradrenergic neurons in the locus ceruleus but not other cells in the surrounding area. Using this antibody, the noradrenergic axons and vesicles were detected in the lumbar region of the spinal cord of nonlesioned rats.

A total of six sections (30 μm sections taken every 5 mm) from the lumbar region of each rat of each treatment group (N - 7 or 8 rats per group) were subjected to morphometric analysis using the Metamorph system (Universal Imaging Corp., West Chester, PA). Fig 2 (Ex. C) shows a region of the spinal cord stained with antibody showing noradrenergic varicosities arranged like beads on axons. The boxed region is a standard area selected for analysis. Fig. 2B shows the varicosities selected in the standard area for computer analysis. The mean number of varicosities

per unit spinal cord area was calculated for each rat group. Figure 2C shows means + SEM for each group. *P < 0.01 for (L + IGF-I) vs. (L + Veh); **P < 0.0002, (L + Veh) vs. NL. These data show that the mean number of adrenergic varicosities was significantly reduced in lesioned rats. Moreover, the mean number of adrenergic varicosities was significantly preserved in IGF-I treated versus vehicle-treated lesioned rats. Thus, subcutaneously administered IGF can act across the blood brain barrier to prevent loss of spinal cord circuitry as well as preserve function in a mammal with a locus ceruleus lesion.

6. Scientific Publications Acknowledge Locus Ceruleus Damage in Neurodegenerative Trauma and Disorders

I have reviewed the scientific literature relating to locus ceruleus damage in neurodegenerative trauma and disorders. The locus ceruleus is involved in many diseases and disorders, which I believe are susceptible to treatment by the invention based on the teachings of the specification and the above-mentioned locus ceruleus model in rat. The following conditions are recognized as involving disease or damage to the locus ceruleus:

(a)	Alzheimer's Disease	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983) (Ex. D); Chan-Palay et al., *J. Comparative Neurology*, 287:373-392 (1989)(Ex. E);
(b)	Parkinson's Disease	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983); Chan-Palay et al., *J. Comparative Neurology*, 287:373-392 (1989);
(c)	Dementia pugilistica	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983);
(d)	Progressive supernuclear palsy	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983);
(e)	Huntington's Disease	Zweig et al., *Annals of Neurology* 24:233-242 (1988) (Ex. F);
(f)	Pick's Disease	Arima et al., *Acta Neuropathologica* 79:629-33, (1990) (Ex. G);

(g)	Major depression	Chan-Palay et al., *J. Comparative Neurology*, 287:373-392 (1989);
(h)	Suicide	Arango et al., *Biol. Psychiatry* 39:112-120 (1996) (Ex. H);
(i)	Schizophrenia	Chan-Palay et al., *J. Comparative Neurology*, 287:373-392 (1989);
(j)	Down's Syndrome	Mann et al., *Clinical Neuropathology*, 2:1-7 (1983);
(k)	Shy-Drager Syndrome	Sima et al., *Clinical Neuropathology* 6:49-54 (1987) (Ex. I);
(l)	Rett Syndrome	Nomura et al., *Brain & Development* 7:334-341 (1985) (Ex. J); and
(m)	Olivopontocerebellar atrophy	Grijalba et al., *J. Neural Transmission* 96:135-42 (1994) (Ex. K).

7. Experiments Further Demonstrating Parenterally-Administered IGFs Crossing the Blood-Brain Barrier

An osmotic minipump containing human IGF-I was implanted subcutaneously in the mid-back region of a rat to continuously deliver human IGF-I. Cerebrospinal fluid (CSF) was removed from the rats prior to treatment (zero time) and after 0.5, 1, 2 and 3 days of human IGF-I infusion. The CSF was subjected to acid-ethanol extraction to dissociate IGF from IGF binding proteins. The human IGF-I in rat CSF was measured using a very sensitive ELISA assay that detected human IGF-I but not human IGF-II, insulin, rat IGF-I, rat IGF-II or rat insulin. The anti-IGF antibody was coupled to horse radish peroxidase, and tetramethylbenzidine substrate was used to produce a colored product that permitted detection of immunoreactive human IGF-I (ihIGF-I) at O.D. 450 nm.

Fig. 3 (Ex. L) shows that human IGF-I was measured in rat CSF after 0.5, 1, 2 and 3 days. It was not detected at 0 days, and the lack of signal confirmed that the assay did not detect rat IGF-I, IGF-II or insulin in rat CSF. These data show that human IGF-I administered subcutaneously can cross the blood brain barrier and enter the CSF.

In the second example, human ^{125}I-IGF-I was injected subcutaneously in a rat. After 2 hours, CSF (100 µl) was carefully withdrawn with a syringe mounted on a micromanipulator after surgical exposure of the atlanto-occipital membrane. Serum was also withdrawn. The treated rat CSF and serum, and other test samples, were subjected to polycrylamide gel electrophoresis in sodium dodecylsulfate-containing (SDS PAGE) gels. This gel separates proteins on the basis of size. Fig. 4 (Ex. M) shows a silver stain of the gel (upper panel) and autoradiogram of the same gel (lower panel). The lanes contained the following samples (from left to right): protein molecular weight markers; BSA buffer used to block potential nonspecific adsorption of radioactive IGF to assay tubes; standard ^{125}I-IGF-I plus CSF from untreated rat (shows normal migration position of standard ^{125}I-IGF-I is not influenced by mixing with CSF); standard ^{125}I-IGF-I plus BSA (shows normal migration position of ^{125}I-IGF-I); radioactive CSF from treated rat (shows ^{125}I-IGF injected subcutaneously crosses the BBB and has size indistinguishable from standard intact ^{125}I-IGF-I); standard nonradioactive human IGF-I (shows position of migration on silver-stained gel at 7.5 kDa size); and radioactive plasma from treated rat (shows ^{125}I-IGF-I administered subcutaneously accumulates in plasma to permit crossing the BBB).

Taken together, these data show that human IGF administered subcutaneously *in vivo* in a mammal can accumulate in serum and cross the blood brain barrier as intact IGF.

8. I further declare that all of these statements based on my knowledge are true and all statements made on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

11-12-98
Date

Dr. Douglas N. Ishii

Please indicate receipt of the ›w-identified paper:

☐ New Application For:		Priority Date:	
☐ Foreign priority already claimed			
☐ Continuation	☐ CIP	☐ Divisional	CPA ☐
☐ Specification: Pages		☐ Drawings: Sheets	
☐ Response to Office Action Dated:			☐ Final Rejection
☒ Other: SUPPLEMENTAL RESPONSE AND DECLARATION OF DOUGLAS N. ISHII			
☐ Assignment Enclosed	☐ Cert. of Timely Mailing		☐ Exp. Mail:

RECEIVED
TECH CENTER 1600/2900
98 NOV 16 AM 10:46

IDENTIFICATION OF APPLICATION

Serial No.: 08/571,802	
Title: Method for Treating Stroke or Traumatic Injury to the Central Nervous System with IGF-I or IGF-II	
Applicant: ISHII	Attorney: KAM/WAA
Client: COLORADO STATE UNIVERSITY RESEARCH FOUNDATION	AWD File No.: CSUA019--1
Mailed: hand-delivered 11/16/98 Filed:	Due Date: --

NO POSTAGE
NECESSARY
IF MAILED
IN THE
UNITED STATES

BUSINESS REPLY MAIL
FIRST-CLASS MAIL PERMIT NO. 8336 HOUSTON TX

POSTAGE WILL BE PAID BY ADDRESSEE

ATTENTION DOCKETING
ARNOLD WHITE DURKEE
P O BOX 4433
HOUSTON TX 77210-9624

RECEIVED
TECH CENTER 1600/2900
98 NOV 16 AM 10:47

RECD A.W.D.
NOV 20 1998

Concise Encyclopedia Biochemistry

Second Edition

Revised and expanded by
Thomas Scott and Mary Eagleson

Walter de Gruyter
Berlin · New York 1988

Concise Encyclopedia of Biochemistry
first published in 1983 based on the original, German language edition,
Brockhaus ABC Biochemie

Translated into English, revised and enlarged by Thomas Scott and Mary Brewer

First Printing, March 1983
Second Printing with corrections, October 1983

Second edition 1988 by

Thomas Scott, Ph. D.
Department of Biochemistry
University of Leeds
Leeds, England

Mary Eagleson, Ph. D.
Berkshire Road
Sandy Hook
Connecticut 06482, USA

CIP-Titelaufnahme der Deutschen Bibliothek

Concise encyclopedia biochemistry / rev. and expanded by Thomas Scott and Mary Eagleson. [Transl. into English, rev. and enl. by Thomas Scott and Mary Brewer]. - 2. ed. - Berlin ; New York : de Gruyter, 1988
Einheitssacht.: Brockhaus-ABC Biochemie (engl.)
Früher u. d. T.: Concise encyclopedia of biochemistry
ISBN 3-11-011625-1
NE: Scott, Thomas [Bearb.]; EST

Library of Congress Cataloging in Publication Data

Brockhaus ABC Biochemie. English.
Concise encyclopedia biochemistry. - English language ed., 2nd ed. / revised and expanded by Thomas Scott and Mary Eagleson.
Translation of: Brockhaus ABC Biochemie. ©1981.
Rev. ed. of: Concise encyclopedia of biochemistry. 1983.
ISBN 0-89925-457-8 (U.S.)
1. Biochemistry-Dictionaries. I. Scott, Thomas, 1935- . II. Eagleson, Mary, 1945- . III. Concise encyclopedia of biochemistry. IV. Title.
[DNLM: 1. Biochemistry-dictionaries. QU 13 B864]
QD415.A25B713 1988
574.19'2'0321-dc19
DNLM/DLC
for Library of Congress

English language edition

STRUCTURAL GENE (DNA)

EARLY RNA TRANSCRIPT

RANSCRIPT (in mRNA)

lA transcript. All pro- ature RNA transcript is translated.

urs or days for release

Endocytosis

Mature storage granule - contains mainly Zn-insulin with C-peptide and ~5% proinsulin

Insulin

Exocytosis (secretion): triggered by increase of cytoplasmic Ca^{2+}

tes/ omplex

plasmic reticulum, Gol- ngerhans. Secretion of

Type 1 or I.-dependent diabetes (IDD) is caused by a lack of functional B cells, resulting in I. insufficiency. IDD classically appears during childhood or adolescence, but can appear at any age; the patients are totally dependent on exogenous I. for survival, and are prone to ketosis. Type 2 or non-I.-dependent diabetes (NIDD) is due to a relative I. deficiency, often related to defective secretion, although synthesis of I. may be adequate. NIDD is more characteristic of middle age and older, and is also known as maturity onset diabetes; exogenous I. is not required, and control by diet alone is often possible. [W. Montague, *Diabetes and the Endocrine Pancreas - A Biochemical Approach* (Croom Helm, London, 1983); M.P. Czech, ed., *Molecular Basis of Insulin Action* (Plenum Press, New York, 1985)]

Insulin-like growth factor, ***IGF, non-suppressible insulin-like activity, NSILA:*** a family of polypeptides present in vertebrate blood that share considerable structural and functional similarity with insulin, but are distinguishable from it by a lack of immunological cross reactivity. Insulin has long-term growth effects, but is more remarkable for its potent short-term action, whereas IGFs show potent long-term effects. Two IGFs (IGF-I and IGF-II) are present in human serum; they possess growth-promoting activity on chick embryo fibroblasts at a concentration of 10^{-9}M.

IGF-I is also called somatomedin C. IGF-II is similar to Multiplication stimulating activity (see) of rat. The structures of human IGF-I and IGF-II and proinsulin show obvious homologies (Fig.) The number of sequence differences between the IGFs and proinsulin suggest that duplication of the common ancestral gene occurred before the appearance of the vertebrates. The three-dimensional structures of insulin and the IGFs are probably similar, since all half-Cys and Gly residues and most of the non-polar core residues of the insulin monomer are conserved.

IGF-I receptor and Insulin receptor (see) are immunologically, structurally and functionally related. IGF-I receptor is a disulfide-linked glycoprotein heterotetramer, M_r about 400000, which can be resolved into α- and β-subunits, M_r 130000 and 98000, respectively. It binds IGF-I with high affinity, IGF-II, multiplication stimulating activity and insulin with lower affinity; it displays Protein-tyrosine kinase (see) activity. IGF-II receptor is a single chain glycoprotein, M_r about 250000, with a high affinity for IGF-II and multiplication stimulating activity, and no affinity for IGF-I or insulin. Insulin can cause a reversible desensitization of the induction of tyrosine aminotransferase by IGF-I or IGF-II, possibly by affecting a post-binding step of IGF action, which may be common to both insulin and IGF. There is evidence that Ca^{2+} has a role in the binding and subsequent cellular processing of IGF-II in pancreatic acini. [C. Thibault et al., *J. Biol. Chem.* **259** (1984) 3361-3367; K.-T. Yu &

Effects of insulin on phosphorylation and activity of various enzymes and proteins. The effects are indirect, i.e. I. binds to its receptor on the cell membrane, initiating a chain of unknown events, culminating in the phosphorylation (kinase action) or dephosphorylation (phosphatase action) of the enzyme.

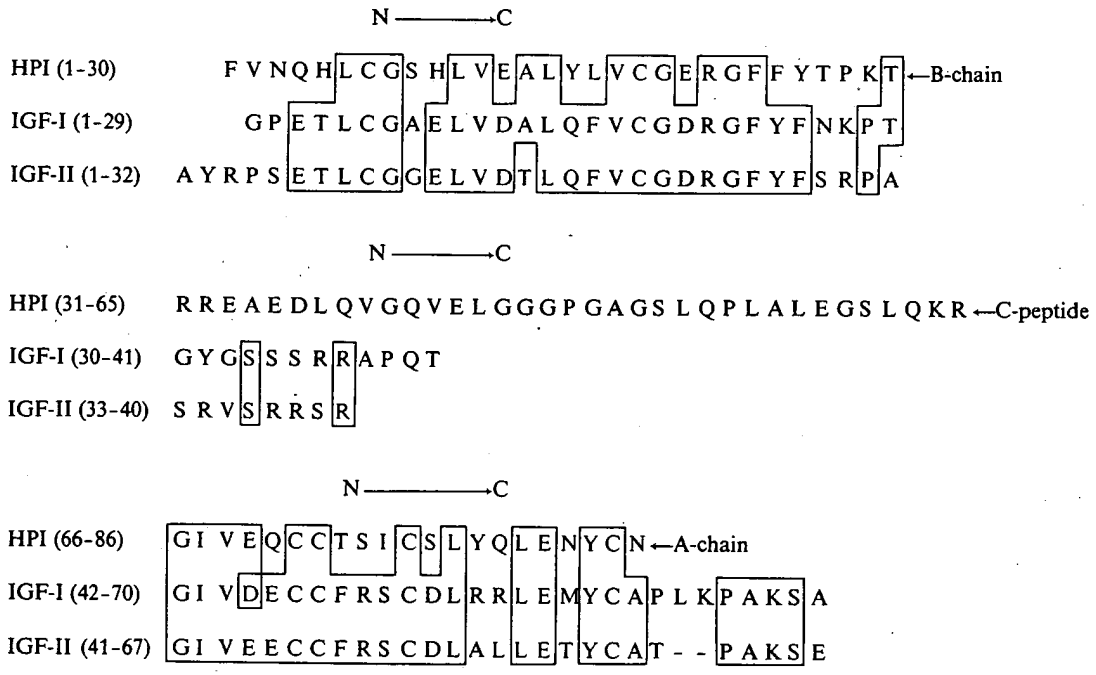
Enzymes of carbohydrate metabolism	Activity	Phosphorylation
Fructose 6-phosphate 2-kinase	↑	↓
Glycogen synthase	↑	↓
Phosphorylase	↓	↓
Phosphorylase kinase	↓	↓
Phosphoprotein phosphatase inhibitor 1	↓	↓
Pyruvate dehydrogenase	↑	↓
Pyruvate kinase	↑	↓
Enzymes of lipid metabolism		
Acetyl CoA carboxylase	↑	↑
ATP-citrate lyase	No change	↑
Diacylglycerol acyltransferase	↑	↓
Glycerol phosphate acyltransferase	↑	↓
Hydroxymethylglutaryl CoA reductase	↑	↓
Hydroxymethylglutaryl CoA reductase kinase	↓	↓
Triacylglycerol lipase	↓	↓
Other		
Insulin receptor (β-subunit)	?	↑
Ribosomal protein S6 (in 40S subunit)	?	↑
Cyclic AMP phosphodiesterase (low K_{III} type)	↑	↑
Ca-ATPase of plasma membrane	↓	↓?
Na/K-ATPase of plasma membrane	↑?	↑

↑ = phosphorylation or increase in activity
↓ = dephosphorylation or decrease in activity

Effects of insulin and other hormones on carbohydrate and lipid metabolism in muscle, adipose tissue and liver. I. stimulates amino acid uptake and protein synthesis in all three tissues.

Tissue	Process	Insulin	Adrenalin	Glucagon
Muscle	Glucose uptake	↑	↑	-
	Glycolysis	↑	↑	-
	Glycogenolysis	↓	↑	-
	Glycogen synthesis	↑	↓	-
Adipose tissue	Glucose uptake	↑	↓	(↓)
	Lipogenesis	↑	↓	(↓)
	Lipolysis	↓	↑	(↑)
Liver	Fatty acid synthesis	↑	↓	↓
	Fatty acid oxidation	↓	↑	↑
	Gluconeogenesis	↓	(-)	↑
	Glycogenolysis	↓	↑	↑
	Glycogen synthesis	↑	↓	↓
	Ketone body formation (ketogenesis)	↓	(-)	↑

↑ = increase in activity; ↓ = decrease in activity



Comparison of the primary structures of IGFs and human proinsulin (HPI). For the one letter code, see Amino acids.

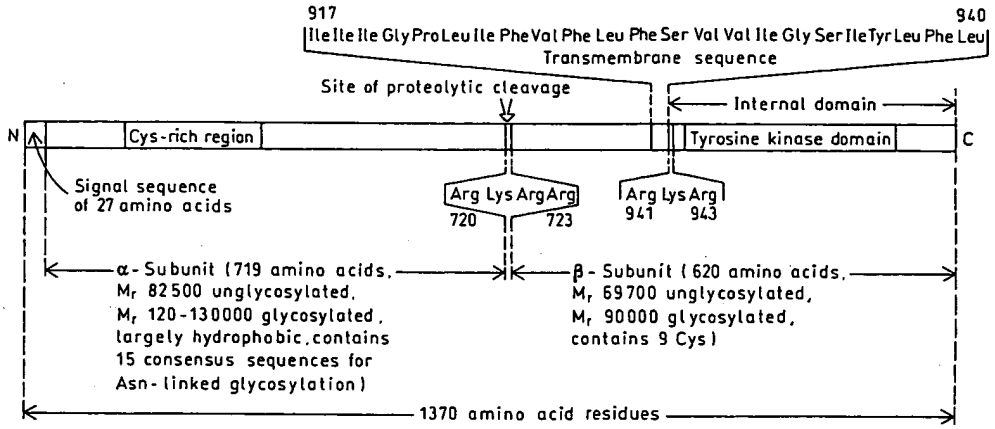


Figure 1. Structure of the precursor polypeptide of the insulin receptor. A sequence of basic amino acids (in this case $Arg_{941}Lys_{942}Arg_{943}$) at the junction of the transmembrane sequence and the cytoplasmic domain is a common feature in the structure of transmembrane proteins. It is thought that they interact with the polar groups of the phospholipids on the membrane surface.

M. P. Czech, *J. Biol. chem.* **259** (1984) 3090–3095; J. Mössner et al., *J. Biol. Chem.* **259** (1984) 12350–12356]

Insulin receptor: The short-term metabolic effects and the long-term growth-promoting activity of insulin are initiated by its binding to specific, high-affinity cell surface receptors. The I.r. is an integral membrane glycoprotein, consisting of 2 α and 2 β subunits linked by disulfide bonds. This heterotetrameric structure is derived from a single polypeptide precursor (Fig. 1), the structure of which was deduced from a cDNA clone. Removal of the signal sequence, partial glycosylation, folding of the polypeptide, and formation of the disulfide linkages (destined to link the α and β subunits) occur in the endoplasmic reticulum. Further glycosylation and proteolytic cleavage into α and β subunits occur in the Golgi apparatus, followed by transport to the plasma membrane. Analysis of the binding of ^{125}I-labelled insulin to the I.r. shows a curvilinear Scatchard plot and a binding constant of 1 nM. Mild reductive cleavage (dithiothreitol) of Triton X-100-solubilized, affinity-labelled I.r. (M_r about 440000) produces identical dimers (M_r about 220000) by cleavage of disulfide bonds between α-subunits. Complete reduction produces separate α and β subunits (M_r about 120000 and 90000, respectively) with high activity of ^{125}I-insulin attached to the α-subunit. Thus each α-subunit binds a molecule of insulin externally to the membrane surface. Only the β-subunits traverse the membrane (Fig. 2). Insulin binding causes the appearance of tyrosine kinase activity (interpreted as a large increase in the V_{max} of an existing active center) in the intracellular domain of the β-subunit. This insulin-dependent kinase catalyses phosphorylation by ATP of Tyr residues in the β-subunit itself, as well as in other peptides and proteins (see Protein-tyrosine kinase). Serine residues of the I.r. also become phosphorylated, but the serine kinase responsible is not intrinsic to the I.r. The significance of Tyr and Ser phosphorylation in the function of the I.r. is not known.

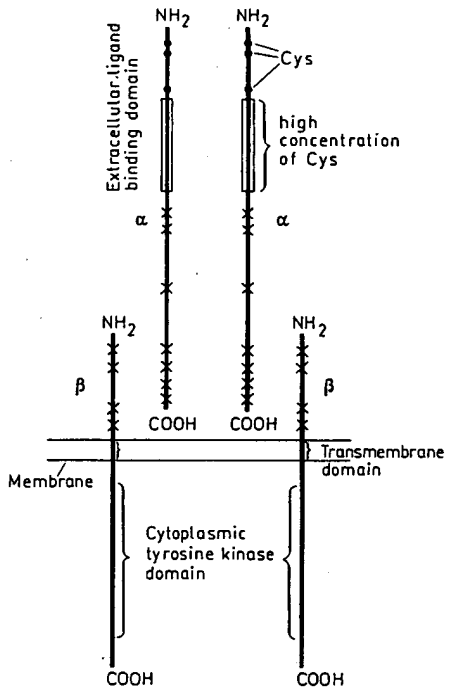


Figure 2. Proposed organization of the heterotetrameric insulin receptor complex. Single Cys residues that may be involved in disulfide linkages between subunits are represented by X.

There are many similarities between the β-subunit of the I.r., the receptors for other growth factors, and certain oncogene products. Thus tyrosine kinase activity is also present in Epidermal growth factor receptor (see), which shows considerable sequence homology with the β-subunit of the I.r., and also